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Crystal structure of glucooligosaccharide oxidase from *Acremonium strictum*

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Introduction

Sugar oxidases and dehydrogenases that catalyze carbohydrate oxidation into the corresponding lactones are of considerable commercial importance. Glucooligosaccharide oxidase (GOOX) from *Acremonium strictum* was screened with the aim of identifying enzymes with potential applications such as an oligosaccharide acid production and various alternative carbohydrate assays. Screening of more than 50 carbohydrates and derivatives showed that D-glucose, maltose, lactose, and cellobiose are good substrates. In addition, this enzyme can react with malto- and cello-oligosaccharides, and hence the name of this novel oxidase. The broad substrate specificity of GOOX, particularly towards oligosaccharides, suggests that it may have great potential applicability.

To facilitate further characterization and the potential industrial use of *A. strictum* GOOX, we have cloned and expressed the encoding gene. Interestingly, even though GOOX shares some substrate specificity with glucose oxidase and cellobiose dehydrogenase, it has no sequence similarity to them. In contrast, GOOX displays significant sequence homology to the FAD-binding domain of plant berberine bridge enzyme-like proteins, particularly the characteristic flavinylation site (RSGGH). To gain structural insights into the FAD incorporation, substrate specificity and catalytic mechanism, we have determined the GOOX structure at 1.55-Å resolution.

Results and Discussion

The protein structure is composed of the FAD-binding domain and the substrate-binding domain (Fig. 1). The F domain consists of two (α + β) subdomains: a small subdomain, comprising a central four β strands (β1-β4), and the second subdomain containing five antiparallel β strands (β5-β9). These two subdomains are surrounded by α-helices, packed against each other, and accommodate the FAD cofactor between them. The S domain possesses a large seven-strand antiparallel β sheet (β10-β16) flanked by five helices and constitutes most of the carbohydrate-binding groove.

Unexpectedly, the FAD cofactor is cross-linked to the enzyme at two attachment sites. One is the Sγ atom of Cys130 bound to the C6 atom of the isoalloxazine ring, while the other is the Nδ1 atom of His70 bound to the 8α-methyl group (6-*S*-cysteinyl, 8α-*N*1-histidyl FAD). This is a novel form of covalent flavinylation; it is the first example of 6-*S*-cysteinyl FAD and is the first double covalent linkage identified to date.

A variety of carbohydrate molecules were modelled into the carbohydrate-binding groove using the Insight II package (Accelrys). The resulting models are consistent with the substrate specificity from kinetic measurements. The results suggest that the most significant enzyme-carbohydrate contacts are clustered at the +1 and +2 subsites, and weaker interactions extend from the +3 to +4 subsites. D-glucose is the only monosaccharide substrate for GOOX. Simulation of the complexes shows that other hexoses and derivatives form either fewer bonds or unfavorable contacts with the amino acid residues surrounding the substrate-binding groove. The non-reducing end of the glucose residue can stick out into the solvent. This explains why, because of the open carbohydrate-binding groove, the enzyme is able to utilize oligosaccharides as good substrates.

Structure-based mutation analysis regarding the flavinylation, substrate specificity and catalytic mechanism, are under investigation.

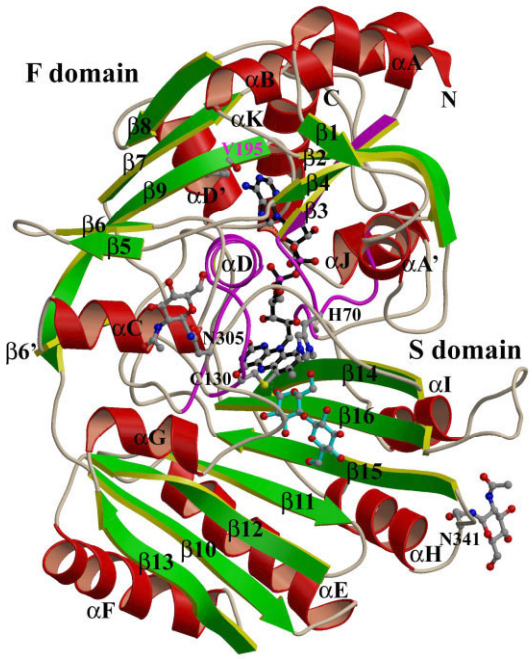

Fig. 1 The GOOX structure. The modeled substrate cellobiose (cyan), the cofactor FAD (black) and the linking residues His70 and Cys130, and the glycosylated Asn305 and Asn341 are displayed as ball-and-stick representations. The Four FAD-interacting segments are highlighted in magenta.

References

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